

CLAIMS

What is claimed is:

1. A method of conveying soybean sudden death resistance into non-resistant soybean germplasm comprising  
5 the step of introgressing soybean sudden death resistance into non-resistant soybean germplasm using one or more nucleic acid markers for marker assisted selection among soybean lines to be used in a soybean breeding programs, said markers associated with soybean sudden death syndrome  
10 resistance.

2. The method of claim 1, wherein said one or more nucleic acid marker is selected from the group consisting of  
OI03<sub>450</sub>, OI03<sub>512</sub>, SATT309, SATT214, SATT275, SIUSAT122,  
CTAAGG280, CGGAGA300, ATGCGA190, AGGCAC310, CCACCA120,  
15 CCCTC220, ACGCAT80, OG13<sub>490</sub>, Bng122, SATT163, SATT38, A112I,  
OE04<sub>450</sub>, OE02<sub>1000</sub>, SATT130, OC01<sub>500</sub>, OO04<sub>1075</sub>, SATT9, OO05<sub>250</sub>,  
K455D, OP13<sub>500</sub>, OG01<sub>1000</sub>, SZ19<sub>500</sub>, SATT71, OW15<sub>1000</sub>, AO85, OA12<sub>1000</sub>,  
BLT65, CCAAGC309, CCCATG349, CCGAAC400, CCGAAC401,  
CCCATG350, CCAAGC310, OW15500, OD04<sub>500</sub>, A063I and SAT40.

20 3. The method of claim 1, wherein said marker assisted selection comprises the use of an analysis technique selected from the group consisting of RAPD analysis, RFLP analysis, microsatellite analysis, and AFLP analysis.

4. The method of claim 1, wherein the source of the sudden death syndrome resistance comprises a soybean strain selected from the group consisting of Forrest or a descendant thereof, Essex or a descendant thereof, Pyramid or a descendant thereof, Ripley or a descendant thereof, Jack or a descendant thereof, PI520733 or a descendant thereof, PI567507B or a descendant thereof, PI567.365 or a descendant thereof, PI567.446B or a descendant thereof, PI567.373B or a descendant thereof, PI90763 or a descendant thereof, PI209332 or a descendant thereof.

5. A method of reliably and predictably introgressing soybean sudden death syndrome resistance into non-resistant soybean germplasm comprising using one or more nucleic acid markers for marker assisted selection among soybean lines to be used in a soybean breeding program, wherein the nucleic acid markers are: OI03<sub>450</sub>, OI03<sub>512</sub>, SATT309, SATT214, SATT275, SIUSAT122, CTAAGG280, CGGAGA300, ATGCGA190, AGGCAC310, CCACCA120, CCCTC220, ACGCAT80, OG13<sub>490</sub>, Bng122, SATT163, SATT38, A112I, OE04<sub>450</sub>, OE02<sub>1000</sub>, SATT130, OC01<sub>500</sub>, OO04<sub>1075</sub>, SATT9, OO05<sub>250</sub>, K455D, OP13<sub>500</sub>, OG01<sub>1000</sub>, SZ19<sub>500</sub>, SATT71, OW15<sub>1000</sub>, AO85, OA12<sub>1000</sub>, BLT65, CCAAGC309, CCCATG349, CCGAAC400, CCGAAC401, CCCATG350, CCAAGC310, OW15500, OD04<sub>500</sub>, A063I and

SAT40, and introgressing said resistance into said non-resistant soybean germplasm.

6. The method of claim 5, wherein said marker assisted selection comprises the use of an analysis technique selected from the group consisting of RAPD analysis, RFLP analysis, microsatellite analysis, and AFLP analysis.

7. The method of claim 5, wherein the source of the sudden death syndrome resistance comprises a soybean strain selected from the group consisting of Forrest or a descendant thereof, Essex or a descendant thereof, Pyramid or a descendant thereof, Ripley or a descendant thereof, Jack or a descendant thereof, PI520733 or a descendant thereof, PI567507B or a descendant thereof, PI567.365 or a descendant thereof, PI567.446B or a descendant thereof, PI567.373B or a descendant thereof, PI90763 or a descendant thereof, and PI209332 or a descendant thereof.

8. A quantitative trait locus associated with resistance to soybean sudden death syndrome, said locus mapping to a linkage group in the soybean genome, said linkage group selected from the group consisting of G, N, C2, B, D, A2, and C1.

9. A quantitative trait locus of claim 8, said locus mapping to linkage group G and mapped by one or more of the

markers OI03<sub>450</sub>, OI03<sub>512</sub>, SATT309, SATT214, SATT275, SIUSAT122,  
CTAAGG280, CGGAGA300, ATGCGA190, AGGCAC310, CCACCA120,  
CCCTC220, ACGCAT80, OG13<sub>490</sub>, Bng122, SATT163, SATT38, A112I,  
OE04<sub>450</sub>, OE02<sub>1000</sub>, and SATT130.

5           10. A quantitative trait locus of claim 8, said locus  
mapping to linkage group N and mapped by one or more of the  
markers OC01<sub>500</sub>, OO04<sub>1075</sub> and SATT9.

10           11. A quantitative trait locus of claim 8, said locus  
mapping to linkage group C2 and mapped by one or more of the  
markers OO05<sub>250</sub>, K455D and OP13<sub>500</sub>.

          12. A quantitative trait locus of claim 8, said locus  
mapped by one or more of the markers OG01<sub>1000</sub>, SZ19<sub>500</sub> and  
SATT71.

15           13. A quantitative trait locus of claim 8, said locus  
mapping to linkage group A2 and mapped by one or more of the  
markers OW15<sub>1000</sub>, AO85, OA12<sub>1000</sub>, BLT65, CCAAGC309, CCCATG349,  
CCGAAC400, CCGAAC401, CCCATG350, CCAAGC310, OW15500, and  
OD04<sub>500</sub>.

20           14. A quantitative trait locus of claim 8, said locus  
mapping to linkage group C1 and mapped by one or more of the  
markers A063I and SAT42.

          15. An isolated and purified genetic marker associated  
with soybean sudden death (SDS) resistance in soybeans, said

marker mapping to a linkage group in the soybean genome, said linkage group selected from the group consisting of G, N, D, C2, B, A2, and C1.

16. The genetic marker of claim 15, further defined as  
5 an isolated and purified polynucleotide comprising a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NOS: 1-20, wherein said polynucleotide hybridizes to the sequence of SEQ ID NOS: 1-20.

10 17. The genetic marker of claim 16, further defined as comprising a base sequence that is identical or complementary to a segment of at least 25 contiguous bases of SEQ ID NOS: 1-20.

15 18. The genetic marker of claim 17, further defined as comprising a base sequence that is identical or complementary to a segment of at least 100 contiguous bases of SEQ ID NOS: 1-20.

19. The genetic marker of claim 18, further defined as having a base sequence that is identical or complementary to  
20 SEQ ID NOS: 1-20.

20. An isolated and purified polynucleotide consisting of 10 to 1500 nucleotides which hybridizes preferentially to RNA or DNA of soybean and not to non-soybean organisms,

wherein said polynucleotide sequence is or is complementary to a nucleotide sequence consisting of at least ten consecutive nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS: 1-20.

5           21. A probe comprising a polynucleotide of claim 20 and a detectable moiety.

          22. An improved soybean plant, seed or tissue culture produced by the method of claim 1.

10           23. An improved soybean plant, seed or tissue culture produced by the method of claim 2.

          24. An improved soybean plant, and parts thereof, which evidences a resistance response to soybean sudden death syndrome (SDS) comprising a genome, homozygous with respect to genetic alleles which are native to a first parent and  
15 nonnative to a second parent of the improved soybean plant, wherein said second parent evidences significantly less resistant response to SDS than said first parent and said improved plant comprises alleles from said first parent that evidences resistance to SDS in hybrid combination in at least  
20 one locus selected from: a locus mapping to linkage group G and mapped by one or more of the markers OI03<sub>450</sub>, OG13<sub>490</sub>, Bng122, SATT163, SATT38, A112I, OE04<sub>450</sub>, OE02<sub>1000</sub> and SATT130; a locus mapping to linkage group N and mapped by one or more

of the markers OC01<sub>500</sub>, OO04<sub>1075</sub> and SATT9; a locus mapping to linkage group C2 and mapped by one or more of the markers OO05<sub>250</sub>, K455D and OP13<sub>500</sub>; a locus mapped by one or more of the markers OG01<sub>1000</sub>, SZ19<sub>500</sub> and SATT71; a locus mapping to linkage group A2 and mapped by one or more of the markers OW15<sub>1000</sub>, A085 and OA12<sub>1000</sub>; or a locus mapping to linkage group C1 and mapped by one or more of the markers A063I and SAT42, said resistance not significantly less than that of the first parent in the same hybrid combination, and yield characteristics which are not significantly different than those of the second parent in the same hybrid combination.

25. The inbred soybean plant of claim 24 comprising each of: a locus mapping to linkage group G and mapped by one or more of the markers OI03<sub>450</sub>, OG13<sub>490</sub>, Bng122, SATT163, SATT38, A112I, OE04<sub>450</sub>, OE02<sub>1000</sub> and SATT130; a locus mapping to linkage group N and mapped by one or more of the markers OC01<sub>500</sub>, OO04<sub>1075</sub> and SATT9; a locus mapping to linkage group C2 and mapped by one or more of the markers OO05<sub>250</sub>, K455D and OP13<sub>500</sub>; a locus mapping to linkage group D and mapped by one or more of the markers OG01<sub>1000</sub>, SZ19<sub>500</sub> and SATT71; a locus mapping to linkage group A2 and mapped by one or more of the markers OW15<sub>1000</sub>, A085 and OA12<sub>1000</sub>; or a locus mapping to linkage group C1 and mapped by one or more of the markers

A063I and SAT42, and having improved resistance to SDS when compared to a substantially identical inbred soybean plant not comprising said loci.

26. A soybean plant, and parts thereof, as claimed in  
5 claim 24 comprising the progeny of a cross between first and  
second inbred lines, alleles conferring resistance to sudden  
death syndrome (SDS) being present in the homozygous state in  
the genome of one or the other or both of said first and  
second inbred lines such that the genome of said first and  
10 second inbreds together donate to the hybrid a complement of  
alleles necessary to confer the resistance to SDS.

27. A sudden death syndrome resistant hybrid and parts  
thereof formed with the improved soybean plant according to  
claim 25.

15 28. A soybean plant and parts thereof formed by selfing  
the sudden death syndrome resistant hybrid of claim 27.

29. A method for the production of an inbred soybean  
plant adapted for conferring, in hybrid combination with a  
suitable second inbred, resistance to sudden death syndrome  
20 (SDS) comprising:

- (a) selecting a first donor parental line possessing  
the desired SDS resistance having at least one of  
the resistant loci selected from: a locus mapping

to linkage group G and mapped by one or more of the markers OI03<sub>450</sub>, OG13<sub>490</sub>, Bng122, SATT163, SATT38, A112I, OE04<sub>450</sub>, OE02<sub>1000</sub> and SATT130; a locus mapping to linkage group N and mapped by one or more of the markers OC01<sub>500</sub>, OO04<sub>1075</sub> and SATT9; a locus mapping to linkage group C2 and mapped by one or more of the markers OO05<sub>250</sub>, K455D and OP13<sub>500</sub>; a locus mapping to linkage group D and mapped by one or more of the markers OG01<sub>1000</sub>, SZ19<sub>500</sub> and SATT71; a locus mapping to linkage group A2 and mapped by one or more of the markers OW15<sub>1000</sub>, A085 and OA12<sub>1000</sub>; or a locus mapping to linkage group C1 and mapped by one or more of the markers A063I and SAT42, and crossing same with a second parental line which is high yielding in hybrid combination, to produce a segregating plant population;

- (b) screening the plant population for identified chromosomal loci of one or more genes associated with the resistance to the SDS trait; and
- (c) selecting plants from said population having said identified chromosomal loci for further screening until a line is obtained which is homozygous for

resistance to SDS at sufficient loci to give  
resistance to SDS in hybrid combination.

30. A method for identifying soybean sudden death  
syndrome (SDS) resistance or soybean cyst nematode resistance  
5 in a soybean plant using a SDS resistance gene, a SCN  
resistance gene or DNA segments having homology to a SDS  
resistance gene or to a SCN resistance gene, the method  
comprising the steps of:

(a) probing nucleic acids obtained from the soybean  
10 plant with a probe derived from said SDS resistance  
gene or from said SCN resistance gene or from said  
DNA segment having homology to said SDS resistance  
gene or to said SCN resistance gene; and

(b) observing hybridization of said probe to said  
15 nucleic acids, the presence of said hybridization  
indicating SDS or SCN resistance in said soybean  
plant.

31. The method of claim 30, further comprising using  
the probe derived from a known resistance gene to probe plant  
20 nucleic acids to look for mapping of the probe to loci for  
SDS or SCN resistance, the presence of said mapping  
indicating the presence of SDS or SCN resistance.

32. A method of reliably and predictably introgressing soybean cyst nematode resistance into non-resistant soybean germplasm comprising using one or more nucleic acid markers for marker assisted selection among soybean lines to be used in a soybean breeding program, wherein the nucleic acid markers are OI03<sub>450</sub>, OG13<sub>490</sub>, Bng122, SATT163, SATT37, OG01<sub>1000</sub> SZ19<sub>500</sub>, SATT71, OW15<sub>1000</sub>, A085 and OA12<sub>1000</sub>, and introgressing said resistance into said non-resistant soybean germplasm.

33. A quantitative trait locus associated with resistance to soybean cyst nematode, said locus mapping to linkage group B and mapped by one or more of the markers OG01<sub>1000</sub> SZ19<sub>500</sub> and SATT71.

34. An improved soybean plant, seed or tissue culture produced by the method of claim 32.

35. An improved soybean plant having soybean cyst nematode (SCN) resistance, the genome of which contains genes associated with said SCN resistance at a locus mapping to linkage group B and mapped by one or more of the markers OG01<sub>1000</sub> SZ19<sub>500</sub> and SATT71.

36. An isolated and purified soybean rfs1 gene, said gene capable of conveying *Fusarium solani*-infection resistance to a non-resistant soybean germplasm, said gene located within a quantitative trait locus mapping to linkage

group G and mapped by genetic markers OI03<sub>512</sub> and Bng122, said gene located along said quantitative trait locus between said markers.

37. An isolated and purified soybean rft1 gene, said gene capable of conveying *Fusarium solani* toxin resistance to a non-resistant soybean germplasm, said gene located within a quantitative trait locus mapping to linkage group G and mapped by genetic markers OI03<sub>512</sub> and SATT309, said gene located along said quantitative trait locus between said markers.

38. A method of determining soybean sudden death syndrome resistance in a soybean plant in a greenhouse setting, the method comprising the steps of:

- (a) inoculating soil with a low density inoculum of *Fusarium solani*;
- (b) planting a soybean plant in said inoculated soil;
- (c) growing said plant in said soil for a predetermined period of time in a greenhouse;
- (d) isolating *Fusarium solani*-infected tissue from said plant;
- (e) culturing said infected tissue for a period of time sufficient to allow for fungal colony forming unit growth;

(f) statistically determining disease severity and/or infection severity in said plant using the number of said fungal colony forming units; and

(g) comparing said disease severity and/or said infection severity to disease severity and/or infection severity data from genetic markers associated with soybean sudden death syndrome resistance to identify a correlation, the presence of a statistically significant correlation indicating the presence of soybean sudden death syndrome resistance in said soybean plant.

39. The method of claim 38, wherein said low density inoculum of *Fusarium solani* comprises an inoculum density of about  $3 \times 10^3$  spore/cm<sup>3</sup> soil.

40. The method of claim 38, wherein said *Fusarium solani*-infected plant tissue comprises root tissue.

41. A method of characterizing resistance to soybean sudden death syndrome in a soybean plant, the method comprising the steps of:

(a) isolating roots from a soybean plant infected by *Fusarium solani*;

- (b) culturing the root on a culture plate including a restrictive growth medium that provides for slow fungal growth and restricted bacterial growth;
- (c) determining root infection severity by statistically evaluating the number of *Fusarium solani* colony forming units on said culture plate; and
- (d) characterizing resistance to soybean sudden death syndrome in said soybean plant based on said determined root infection severity.

42. The method of claim 41, wherein said characterization of resistance to soybean sudden death syndrome further comprises determining a level of resistance to soybean sudden death in the soybean plant.